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Effects of Manganese and Their Modification by Hexametaphosphate

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The ability of oral Mn^{++} to produce the depletions of dopamine in the corpus striata characteristic of the Parkinson-like syndrome in manganese workers was examined in rats. Mn^{++} failed to affect dopamine levels at concentrations of up to 2000 mg/liter of drinking water and six months of continuous exposure.

A second objective of this work was to study the biological interactions between Mn^{++} and sodium hexametaphosphate [$(NaPO_3)_6$] administered simultaneously in drinking water. Some evidence was obtained that high concentrations of $(NaPO_3)_6$ depleted tissue Mn^{++} and produced decreased body weight gains over the experimental period. Similarly, Mn^{++} in high concentrations resulted in decreased growth of weanling animals. Stoichiometric combinations of Mn^{++} and $(NaPO_3)_6$ prevented the decreased rate of growth produced by either agent alone.

On the basis of these results, we conclude that Mn^{++} in drinking water presents little hazard in terms of central nervous system involvement in a homogeneous population. We cannot, however, exclude the involvement of genetic factors which have been suggested to account for the sporadic occurrence of Parkinson-like symptoms in Mn^{++} workers. The use of $(NaPO_3)_6$ for Mn^{++} complexation as an alternative to removal in drinking water treatment appears safe from a health standpoint if carefully controlled to avoid excessive amounts of $(NaPO_3)_6$.

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EFFECTS OF MANGANESE AND THEIR MODIFICATION BY HEXAMETAPHOSPHATE

by

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. In Cincinnati, the Environmental Research Center possesses this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and

- a search for ways to prevent contamination and to recycle valuable resources.

The Health Effects Research Laboratory conducts studies to identify environmental contaminants singly or in combination, discern their relationships, and to detect, define, and quantify their health and economic effects utilizing appropriate clinical, epidemiological, toxicological, and socio-economic assessment methodologies.

Source waters for public drinking water supplies occasionally contain quite high concentrations of manganese. Although this element is essential for normal health, inhalation of high concentrations of manganese has been shown to result in a syndrome quite similar to Parkinson's disease. The current drinking water standard for manganese was established because of certain esthetic problems associated with its presence which can be ameliorated by complexation of manganese with certain polyphosphate compounds. Using this form of water treatment does not remove manganese from the water. The present study was undertaken to determine if manganese in drinking water could produce the changes which occur in Parkinson's disease and to evaluate the practice of marganese complexation in water treatment.

R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

Charles River strain male rats were exposed to Mn^{++} concentrations of 50, 200, 700, and 2000 mg/liter in their drinking water for 6 months. A second series of animals were exposed to sodium hexametaphosphate $(NaPO_3)_6$ at concentrations of 185, 742, 2967 and 7420 mg/liter. These stoichiometric concentrations of Mn^{++} and $(NaPO_3)_6$ were combined in the drinking water of a third series of animals.

The primary intent of the study was to determine if Mn^{++} via the oral route could produce decreases in dopamine concentrations of the corpus striata characteristic of the Parkinson-like disease observed in manganese workers and to correlate such changes with Mn content of the tissue. Mn^{++} failed to produce a decrease in corpus striatal dopamine content. However, a paradoxical decrease in corpus striatal Mn concentration was observed at 200 and 700 mg Mn^{++}/liter of drinking water. At 2000 mg Mn^{++}/liter a tendency for corpus striatal Mn to increase was observed, although they did not exceed control levels even at this dosage level. Drinking water containing $(NaPO_3)_6$ resulted in a dose related decrease in corpus striatal Mn concentrations. Mn^{++} and $(NaPO_3)_6$ combinations resulted in a smaller decrease in corpus striatal Mn, although there was no clear evidence of a trend with dosage in the data.

Both Mn^{++} and $(NaPO_3)_6$ alone produced decreased body weight gains which appeared associated with decreased appetite at high concentrations. Lower concentrations of Mn^{++} (50 mg/liter) resulted in an enhanced rate of early growth. Stoichiometric combinations of Mn^{++} and $(NaPO_3)_6$ prevented the decreased rate of growth produced by each agent alone.

We conclude that Mn^{++} in drinking water presents little danger in terms of central nervous system involvement in a homogeneous population. We cannot, however, exclude the involvement of genetic factors which have been suggested to account for the sporadic occurrence of Parkinson-like symptoms in manganese workers. The use of $(NaPO_3)_6$ for Mn complexation as an alternative to removal in drinking water treatment may be feasible if carefully controlled to avoid excessive amounts.

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SECTION I

INTRODUCTION

Toxicity as a result of excessive exposures to manganese has been observed in occupational settings. A syndrome clinically indistinguishable from Parkinson's disease has been reported in mining operations (Mena, et al., 1967), processing of manganese ore (Cook, et al., 1974) and in ferromanganese alloy production (Tanaka & Lieben, 1969; Jonderko, et al., 1971; Smyth, et al., 1971). As in Parkinson's disease, chronic manganism results in substantial depletion of brain dopamine concentrations (Ehringer and Hornykiewicz, 1960; Mustafa and Chandra, 1971) and often responds to treatment with the dopamine precursor L-dihydroxyphenylalamine (Cotzias, et al., 1971; Goldman, 1972).

Manganese has also been shown to be an essential element (Cotzias, 1958). Although frank deficiency in humans has never been reported, the concentration of the metal in the modern diet has probably been reduced by food processing (Schroeder, et al., 1966).

The USEPA drinking water standard for manganese has been established for esthetic reasons at 0.05 mg/liter. Esthetic objectives to manganese in public water supplies may be controlled by complexation with various polyphosphate compounds. It could be argued that if the esthetic requirements of the drinking water standards can be safely managed by complexation, an elevated manganese concentration in drinking water could be tolerated or may even be desirable.

A recent paper (Bonilla & Diez-Ewald, 1974) indicated that brain dopamine levels were reduced by about 50% in rats drinking water containing 5 mg $MnCl_2$ per ml for 7 months. The present study was undertaken to develop dose-response information on this effect of manganese and to determine if complexation with sodium hexametaphosphate modified the effect in any way.

SECTION II

CONCLUSIONS

The drinking water standard for Mn currently in force was established for esthetic reasons. Knowledge of this fact has led certain water treatment facilities to employ complexation of Mn with polyphosphate compounds as an alternative to Mn removal from raw water. This does satisfy esthetic objections to Mn in drinking water. However, a question remains as to what limit should be placed upon Mn concentrations in drinking water from a health standpoint. Secondary questions arise as to the toxic properties of the complexing agent and the complex itself.

Certain tentative conclusions may be arrived at on the basis of the present study. The most severe toxic effect of chronic manganism involves the production of a syndrome which is clinically indistinguishable from Parkinson's disease. In human cases of the disease, this syndrome has been shown to be associated with substantial decreases in brain dopamine content. Similarly, injections of Mn, and in one study, oral ingestion of Mn^{++} have been shown to produce a decrease in brain dopamine concentrations. We observe no such decrease in our experimental animals with exposures of up to 2000 mg Mn^{++}/liter for 6 months. Therefore, we can conclude that orally administered Mn^{++} does not appear a substantial hazard in this regard in a homogeneous population such as our test animals. We cannot, however, exclude the possibility, mentioned in the literature, that a substantial minority of the human population may be susceptible to lower concentrations of Mn^{++} on a genetic basis. Our work indicated a dynamic regulation of tissue Mn^{++} concentrations in the face of widely differing environmental exposures. A defect in this regulation produced by either environmental or genetic factors could dramatically increase sensitivity to Mn^{++}.

The present study did not reveal any overwhelming reasons for precluding the practice of Mn^{++} complexation in treatment of drinking water. The results do suggest that care should be exercised in the use of polyphosphates for this and other purposes. At concentrations of 742 mg $(NaPO_3)_6$ and above frank depletion of tissue Mn was evident. Undoubtedly, the absorption of other trace nutrients were similarly affected. Trends in the data presented on growth of the animals suggested that concentrations lower than 742 mg $(NaPO_3)_6$ may have some impact on health over the long term. Mn^{++} and $(NaPO_3)_6$ mutually antagonized one another's effects on body weight gains. Consequently, the practice of complexation by polyphosphates has some merit if the water treatment dosage is carefully titrated

to avoid addition to excessive polyphosphate. Some note should be added to limit this practice to realistic concentrations of manganese, perhaps 1-2 mg/liter. Even in combination there were some indications of depleted tissue Mn concentrations. Additionally, the mutual antagonism of the two compounds broke down, perhaps due to the toxicity of the complex, at their highest concentrations in combination.

SECTION III

RECOMMENDATIONS

1. The present drinking water standard for manganese should be maintained at 0.05 mg/liter for esthetic reasons.

2. Complexation of manganese present in raw water by sodium hexametaphosphate be accepted as conforming to the above standard as long as the treatment plant confirms on a routine basis that the amount of sodium hexametaphosphate does not exceed the requirement by more than 10% and that manganese concentrations being treated do not exceed 1 mg/liter.

3. The effects of manganese on neonatal animals requires further study. Some limited evidence indicates that mothers subjected to manganese loading excrete large amounts of manganese in their milk. It is possible that less developed mechanisms for controlling absorption, distribution and excretion of manganese in younger animals may result in adverse effects at much lower concentrations. Because of the association of chronic manganism with effects on the control nervous system any studies initiated in this area should emphasize behavioral, neurochemical and neurophysiological parameters.

4. Polyphosphate compounds commonly used in water treatment should also be evaluated experimentally. Data derived wholly from a single compound, sodium hexametaphosphate, is clearly too little with which to extrapolate to an entire class of compounds. It is possible that certain of these compounds could enhance absorption of the offending metal or that the complex itself may possess unique toxicological properties.

5. The biological behavior of other metal complexes with polyphosphate compounds should also be studied. Polyphosphate compounds are apparently used occasionally when iron is present in raw water at concentrations in excess of the drinking water standard. Behavior of a manganese complex does not necessarily extrapolate to other metals. The possibility that polyphosphates may enhance the leaching of toxic metals from the distribution system and/or increase their bioavailability should also be entertained.

SECTION IV

IMPLICATIONS FOR WATER SUPPLY PRACTICE

One of the basic reasons for undertaking the present study was to examine the practice of utilizing polyphosphate compounds for water treatment. This treatment is generally applied to meet certain formal or informal effluent requirements for drinking water. In contrast to the necessity for actual removal of chemicals which are known for their potential as health hazards, this method of treatment is symptomatic and does not involve removal of the offending agent. Rather a complex is formed which remains in the drinking water. Thus one is faced with a three-fold problem when considering possible hazards to the health of populations consuming water treated in such a manner. First, some hazard may be associated with the offending chemical. Second, the hazard associated with the treatment chemical must be evaluated. Third, the complex formed may have distinct toxicological problems of its own or the toxicity of one or both of the parent compounds may be altered by changes in absorption, distribution or excretion.

Before this type of treatment is even contemplated it must be established that the offending chemical is relatively non-toxic. Treatment failure or improper treatment would be too great a risk to assume on a daily basis. For example, such treatment would have to be ruled out *a priori* for such toxic metals as Pb, Cd, and Hg. The present study and prior studies have indicated that manganese fits into the classification of a chemical with relatively low toxicity via the oral route, although certain reservations must be acknowledged in terms of manganese toxicity in young animals (see recommendations). The polyphosphate compounds ordinarily used in water treatment also have been regarded as being relatively safe chemicals in terms of human health. The present study bears this out to a large extent. However, evidence was obtained that high levels of polyphosphates interfered with essential trace metal metabolism. More alarming was a trend that developed in the same direction at lower concentrations as well even though the individual results were not statistically significant. The fact that addition of stoichiometric concentrations of manganese to the drinking water resulted in a significant enhancement of growth rate at 742 mg $(NaPO_3)_6$/liter suggests that this concentration does interfere with trace metal metabolism. Such an effect may have longer-term nutritional consequences in a human population where individual diets vary quite widely. Relatively small concentrations of polyphosphates in the drinking water may be sufficient to induce deficiencies in individuals consuming a diet marginal in essential trace metals.

Since both manganese and sodium hexametaphosphate do produce some signs of toxicity at high concentrations, it is necessary that some limits be established for manganese complexation as a water treatment practice. Both manganese and sodium hexametaphosphate alone appeared more toxic than the complex of the two. Consequently, if the treatment is used it is important that use of sodium hexametaphosphate be carefully adjusted to the requirement posed by manganese in the source water. Barring a source water which varies widely and unpredictably, a water treatment plant with routine analytical capabilities for trace metal analysis should have little difficulty in maintaining a sodium hexametaphosphate dosage rate which does not exceed the stoichiometric manganese content by more than 10%. Manganese at a concentration of 700 mg/liter did not depress growth relative to control animals. However, since lower concentrations resulted in an enhancement of early growth and addition of a stoichiometric quantity of $(NaPO_3)_6$ to water containing 700 mg Mn^{++}/liter significantly enhanced growth, it is apparent that this concentration of manganese was beyond the optimum manganese intake, even though no long term effects appears to result from this level, the pattern dictates a conservative view until the effects of manganese loading can be more fully established in neonatal animals. Taking, therefore, a concentration of 200 mg Mn^{++}/liter as a "no effect" level, use of polyphosphate treatment for concentrations of manganese up to 1 mg/liter in raw water should present little hazard to health. This would not more than double the average manganese present in the human diet assuming a 2 liter daily consumption. A safety factor of 200 would then apply in terms of health even in the event of complete treatment failure. Similarly, if one operates within the above stated restriction of not exceeding the requirement for sodium hexametaphosphate by 10% not more than 4 mg $(NaPO_3)_6$/liter would be added leaving an excess of 0.4 mg of non-complexed $(NaPO_3)_6$/liter. Concentrations of 185 mg $(NaPO_3)_6$/liter would without significant effect on tissue manganese content and growth. This provides a safety factor for non-complexed $(NaPO_3)_6$ of 462. If no metals were present to complex $(NaPO_3)_6$ the safety factor would be reduced to 46 in the long-term. Short-term overdosage should present little hazard. The safety factor for the Mn^{++} plus $(NaPO_3)_6$ complex would be 700 on the basis of present experiments.

It should be noted that these safety factors have been calculated in terms of concentration rather than dose/unit body weight as is ordinarily done. Interactions between polyphosphates and trace metals appear to involve chemical interactions within the gastrointestinal tract and would be expected to be more closely related to concentration than to dose/unit body weight expected of a systemic physiological interaction between the two chemicals. However, since man consumes less water per unit of body weight than rats, the safety factors in terms of dose/unit body weight would be somewhat greater than indicated above.

In conclusion, sodium hexametaphosphate, and possibly other polyphosphate compounds, can be used without undue risk to satisfy esthetic standards for manganese in drinking water. The use of polyphosphates for the purpose of removing esthetic objections arising from the presence of metal ions, however, must be governed by the amount of the offending metal present.

Excess polyphosphate may prevent absorption of essential trace metals from the diet and thus cannot be considered completely inert from a public health standpoint. Consequently, concentrations of polyphosphate greatly in excess of a metal ion defined requirement should be avoided as a questionable public health practice that may have long-term nutritional repercussions.

Therefore, a recommendation is made that the practice of complexing manganese to meet the EPA drinking water standard be restricted to concentrations of Mn which do not exceed 1 mg/liter. In addition, the dosage rate for sodium hexametaphosphate should not exceed the metal ion requirement by more than 10 as defined by routine analysis of the raw water.

SECTION V

METHODS

Male, weanling Charles River Strain rats (Sprague-Dawley) were distributed into 12 experimental groups of 9 animals each. The drinking water (distilled) contained either $MnCl_2$, $MnCl_2$ in stoichiometric combination with sodium hexametaphosphate ($(NaPO_3)_6$), or $(NaPO_3)_6$ alone as shown in Table 1.

TABLE 1. CONCENTRATIONS OF Mn^{++} and/or $(NaPO_3)_6$ UTILIZED

	Mn^{++}	Mn^{++} + $(NaPO_3)_6$	$(NaPO_3)_6$
Mn^{++}	50 mg/liter	50 mg/liter	
$(NaPO_3)_6$		185 mg/liter	185 mg/liter
Mn^{++}	200	200	
$(NaPO_3)_6$		742	742
Mn^{++}	700	700	
$(NaPO_3)_6$		2957	2957
Mn^{++}	2000	2000	
$(NaPO_3)_6$		7420	7420

A control group of 18 animals was maintained on distilled water. All animals were allowed free access to commercial laboratory chow (Teklad, stated Mn^{++} concentration 91 ug/g). Food and water consumption were monitored and animals were periodically weighed throughout the study.

At the end of 6 months, animals were sacrificed, the brains rapidly removed and placed in a cold box. The corpus striatum was dissected out and forzen on a block of dry ice. The pair of striata from each animal were then weighed and homogenized in 0.1 N perchloric acid (60 volumes).

Extracts were centrifuged at 10,000 X g for 10 min and the supernatant decanted. 2.0 ml was taken from dopamine analysis and the remainder was utilized for analysis of manganese content. The samples were preserved at -90° C until analysis could be performed. Sacrifice of animals was scheduled so that one-half of the control animals (9) were the first and the other half the last animals sacrificed to eliminate artifactual trends in the analyses.

A radioassay which made use of the O-methylation reaction of catechol-O-methyl transferase with S-adenosylmethionine (methyl-^{3}H) (6.8-12.6 C$_i$/mM) was utilized for dopamine analysis. The procedure was a modification of that described by Coyle and Henry (1973).

Manganese determinations were made directly following neutralization and removal of perchloric acid by K_2CO_3 by non-flame atomic absorption spectroscopy using a Varian AA-6 fitted with a model 63 carbon rod atomizer. Calculations were made from standard curves obtained from known concentrations of manganese dissolved in 0.1 N perchloric acid. Tissue analysis of both manganese and dopamine were performed blind. The data was tested for significance by analysis of variance and student's t-test.

SECTION VI

RESULTS

The condition of all animals in the study was generally good. No evidence of gross neurological damage was apparent in any experimental group.

The levels of dopamine in the corpus striata of rats on the study did not vary systematically with dose of either Mn^{++} or $(NaPO_3)_6$ (Table II). Corpus striatal dopamine concentrations did exhibit differences which were statistically different from control in certain groups. The maximum deviations were an apparent 17% decrease of dopamine in the 742 mg $(NaPO_3)_6$/liter group and an apparent 13% increase in the group receiving 2000 mg Mn^{++}/liter. These changes were not dose-related and are small enough to be of questionable biological significance.

Manganese content of the corpus striatum exhibited a paradoxical decrease with increasing intake of Mn^{++} (Fig. 1). This amounted to a 50% decrease at 200 mg Mn^{++}/liter of drinking water. A gradual increase of corpus striatal manganese content, towards control levels, was observed at higher concentrations of manganese.

Corpus striatal manganese was also found to decrease with increasing concentrations of $(NaPO_3)_6$ in the drinking water (Fig. 1). The decrease was dose related, but appeared to be maximal at 2942 mg $(NaPO_3)_6$/liter. Chemically equivalent concentrations of Mn^{++} and $(NaPO_3)_6$ resulted in an average decrease in corpus striatal manganese of about 30%. However, there appeared to be no trend with dose. There was no significant correlation between the individual concentrations of manganese and dopamine in corpus striata ($r = 0.034$).

Animals on the different treatments did exhibit significant differences in growth and final body weights relative to control animals (Fig. 2). Inclusion of 50 mg Mn^{++}/liter of drinking water resulted in increased body weights. Although not statistically significant at termination of the study, the difference apparently resulted from a small but significant enhancement of early growth. At the start of the experiment this group averaged 57.1 ± 1.6 g compared to 55.0 ± 1.9 (SEM) for the control group. After 51 days on the study the body weights of these groups were 438 ± 9 and 412 ± 9, respectively ($P = 0.05$). At intermediate concentrations of manganese this difference from the control group were not apparent. Manganese at a concentration of 2000 mg/liter resulted in a significant depression in growth rate, which was already apparent 3 weeks into the study. Despite

the early appearance of growth inhibition only a 10% decrement was observed from control at the end of 6 months and no deaths were observed.

TABLE 2. CORPUS STRIATAL DOPAMINE CONCENTRATIONS IN RATS EXPOSED TO MANGANESE AND/OR SODIUM HEXAMETAPHOSPHATE FOR 6 MONTHS

$(NaPO_3)_6$ mg/l	Mn^{++}, mg/l 0	50	200	700	2000
0	11.2 ± 0.4*	10.0 ± 0.3	11.1 ± 0.4	10.7 ± 0.5	12.8 ± 0.3
185	11.3 ± 0.3	10.7 ± 0.6	-	-	-
742	9.3 ± 0.4	-	9.8 ± 0.4	-	-
2957	12.5 ± 0.5	-	-	12.1 ± 0.3	-
7420	11.8 ± 0.4	-	-	-	10.6 ± 0.3

*µg dopamine/g tissue ± S.E.M.

Sodium hexametaphosphate $(NaPO_3)_6$ was also observed to depress body weight at high concentrations (Fig 3). Unlike manganese, effects on body weight did not become apparent for two months even at 7420 mg $(NaPO_3)_6$/liter. Effects of lower concentrations of $(NaPO_3)_6$ on body weight appeared to be characterized by later departures from the control growth curve. This trend was observed at all concentrations, suggesting that continued exposure may have resulted in significant differences from control at concentrations below 2957 mg/liter, the lowest dose to show significant differences in the present study.

Stoichiometric combinations of Mn^{++} and $(NaPO_3)_6$ indicated that the two substances were basically antagonistic in their effects upon growth rate (Fig 4). The combination of 700 mg Mn^{++} and 2957 mg $(NaPO_3)_6$/liter resulted in an enhancement of body weight gain relative to control at 6 months ($P < 0.05$). The latency of this effect resembled that of $(NaPO_3)_6$ alone as opposed to Mn^{++} alone; not becoming evident until the end of the second month of the study. Combination of 2000 mg Mn^{++} with 7420 mg $(NaPO_3)_6$/liter of drinking water resulted in a small depression of body weight at 6 months. Although not significantly different from the control group ($0.05 < P < 0.1$), it did represent a substantial depression when compared with the group receiving 700 mg Mn^{++} and 2940 mg $(NaPO_3)_6$/liter ($P < 0.01$). A summary of final body weights for each experimental group is provided in Fig 5.

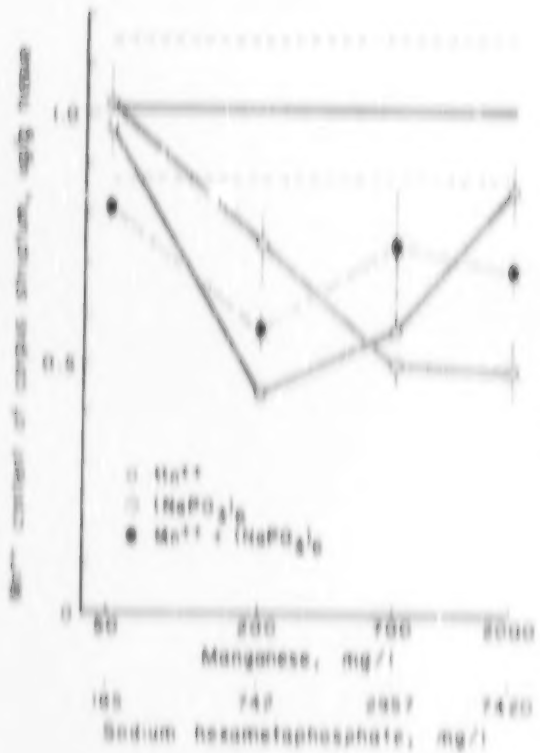

Figure 1. Effects of treatments upon the manganese content of the rats corpus striatum at 6 months of exposure. Horizonal lines indicate the average (solid) ± SEM (dashed) of the control group. All other points show the average ± of the indicated treatment group.

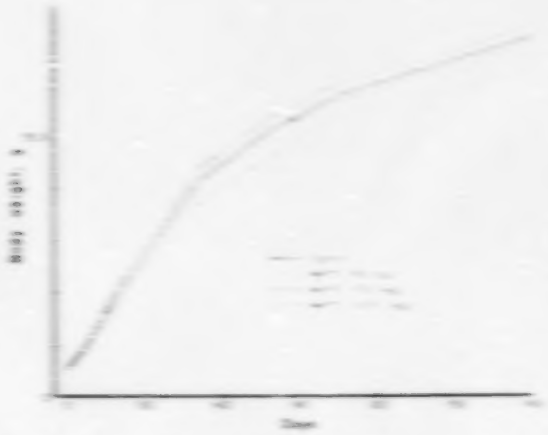

Figure 2. Effects of manganese in drinking water on growth of weanling rats. The 200 µg Mn^{++}/liter group was omitted because it coincided with the control group.

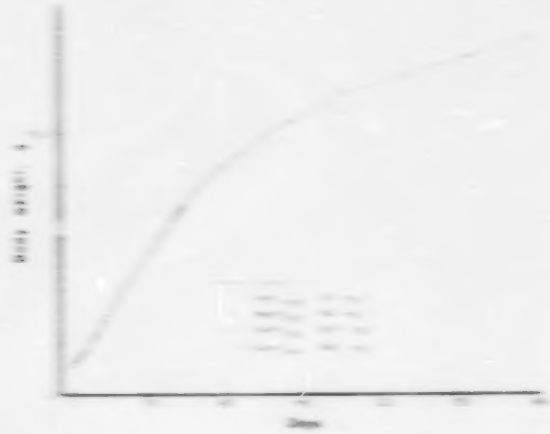

Figure 3. Depression of body weight gain by weanling rats produced by sodium hexametaphosphate in drinking water.

Figure 4. Effects of stoichiometric combinations of Mn^{++} and $(NaPO_3)_6$ on body weight gain of weanling rats.

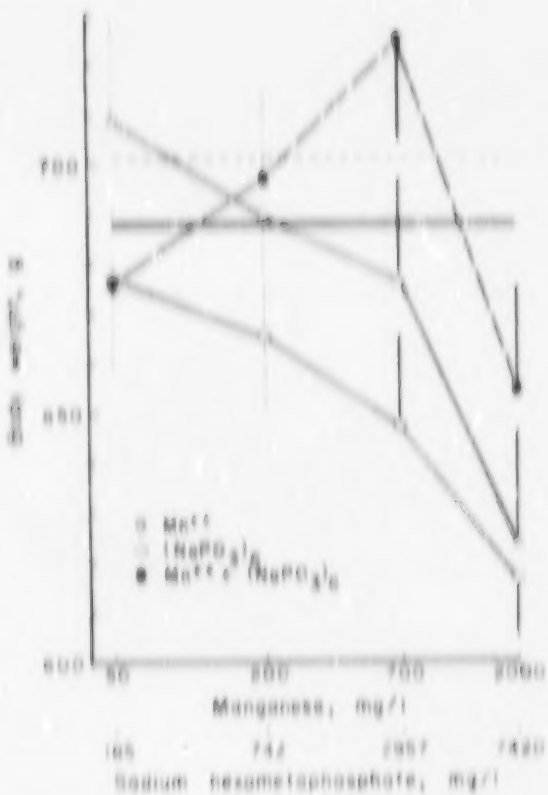

Figure 5. Summary of treatment effects on final body weights. Solid horizontal line represents the average weight of 18 control animals with dashed lines showing the SEM. All other points show the average ± SEM of the indicated treatment group.

Depressions in growth produced by both Mn^{++} and $(NaPO_3)_6$ were associated with decreases in food consumption (data not shown). The latency of decreases in food consumption were very closely associated with the periods in which differences in body weight were first noted. A 10% decrease in the consumption of chow was observed with 2000 mg Mn^{++}/liter during the third week of the study whereas consumption of food by the 7420 mg $(NaPO_3)_6$/liter group was 4% above control. During the sixth week, however, the latter group's consumption of laboratory chow decreased by 10% relative to controls and remained at this level through the twelfth week of the study. Beyond this point, food consumption by this group was consistently below controls but only by 3-5%. Conversely, groups which experienced better weight gains than controls evidenced significantly higher food consumpton which coincided in latency with divergence of body weights from controls. Animals drinking water containing 50 mg Mn^{++}/liter consumed 6.1% more chow than controls for the first 4 weeks of the study. This difference had disappeared by the eighth week. Drinking water containing 700 mg Mn^{++} and 2952 mg $(NaPO_3)_6$/liter exhibited a 5% increase in food consumption during weeks 10-20. The increased growth rate of this group became evident that the day 80 weighing.

SECTION VII

DISCUSSION

The principle purpose of this study was to determine if oral manganese exposures might result in neurological damage similar to that observed in occupational settings which primarily involve inhalation exposures (Mena, et al., 1967; Cook, et al., 1974; Tanaka & Lieben, 1969; Jonerko, et al., 1971; Smyth, et al., 1973). The data reported here indicates that substantial intake of Mn^{++} (up to 100-200 mg/Kg per day for a 6 month period) do not produce the characteristic decrease in dopamine associated with chronic manganism in the male rat. The few differences observed between experimental groups appeared to result from random variation. These data support the conclusion that the drinking water standard for manganese (0.05 mg/liter) is more than adequate to protect against this effect of manganese. The results are different from those of Bonilla and Diez-Ewald (1974). Their study utilized a single exposure level, equivalent to 2182 mg Mn^{++}/liter, for a 7 month period. These authors employed female rats, which might explain the difference in results. Some caution must be exercised to avoid overinterpretation of our negative results, however. Occurence of chronic manganism in exposed populations is not simply a function of concentration. The fact that the disease often develops within a few years in certain individuals but never occurs in other individuals working under the same conditions for much longer periods has led to a suspicion that other factors, such as nutritional status (Mena et al., 1969) or genetic factors (Cotzias, et al., 1972), are necessary determinants of the disease. Consequently, studies in genetically homogeneous animals with nutritionally adequate diets may not completely define the risk. In addition, manganese loads are known to produce changes in carbohydrate and lipid metabolism which could have implications in chronic disease states outside the scope of the present study.

The various treatments did impact on the corpus striatal manganese concentrations. Most interesting was the paradoxical decrease in the tissue's manganese content at 200 mg Mn^{++}/liter of drinking water. While there is no specifically stated precedent for a decrease with increased exposures, some data appears in a paper from Cotzias' laboratory (Hughes et al., 1966) indicating a similar decrease of manganese concentrations in the diaphragm of mice exposed to 20 mg Mn^{++}/liter in a milk diet for 54 days. Excretion of manganese is known to be very sensitive to manganese load, both in humans (Bory and Cotzias, 1958) and experimental animals (Britton and Cotzias, 1966). Distribution of manganese can be influenced by adrenocortical hormones (Hughes et al., 1966) and is known to redistribute with loading

(Papavasiliou et al., 1966). To explain an actual decrease in a tissue concentration in the face of increased exposure to the metal would require active regulation and could not be explained merely by increased excretion due to mass action. Obviously, this potentially important result must be confirmed and coupled with more extensive documentation of manganese distribution with varying manganese loads. Nevertheless, it is apparent that corpus striatal content of manganese is regulated within very close limits and even extreme changes in oral intake have relatively small impacts on the actual tissue concentrations. The tendency for corpus striatal manganese to gradually increase with concentrations above 200 mg Mn^{++}/liter suggests that higher concentrations may have resulted in actual elevations in the tissue's content of the metal. Elevations of whole brain manganese concentrations have been documented in lambs receiving 4030 ppm Mn in the diet (Watson et al., 1973).

The dose-related depletion of corpus striatal manganese with $(NaPO_3)_6$ may be presumably explained by the propensity of this chemical to complex manganese making it less available for absorption. High phosphate to calcium ratios are known to limit the absorption of manganese (Underwood, 1971). The effect of stoichiometric combinations of Mn^{++} and $(NaPO_3)_6$ on corpus striatal manganese concentrations are not so easily understood. Although the curve obtained with the concentration of the combination tends to possess some of the characteristics of that observed with Mn^{++} alone, the trends are less clear. Possibly interactions of Mn^{++} and $(NaPO_3)_6$ with the metabolism of other essential elements may be involved (Mena, et al., 1969; Watson, et al., 1973).

Interactions of Mn^{++} and $(NaPO_3)_6$ were better defined by the effects of the differing treatments of growth. In all cases, the effects of the treatments appeared to exerted through changes in appetite rather than direct toxicity of the chemicals. The optimum intake of manganese varies significantly depending upon the parameter being studied (Underwood, 1971). Holtkamp and Hill (1950) indicated that increasing manganese intake to 2 mg/day was optimal for growth of rats around 30 days of age. Rats on the present study consumed an average of 17 g of chow per day during this period. At 94 ug Mn/g, the manganese in the diet amounted to 1.6 mg/rat per day. Consequently, it is reasonable to conclude that the increased rate of body weight gain in animals consuming 50 mg Mn^{++}/liter in their drinking water resulted from the nutritional contribution. The appetite depressant effects of 2000 mg Mn^{++}/liter and the associated decreases in body weight gain are consistent with the results of previous studies as well (Underwood, 1971). Sodium hexametaphosphate, in contrast to Mn^{++}, possessed only a depressant effect on body weight.

The slightly less than optimal manganese for growth present in the laboratory chow resulted in interactions between Mn^{++} and $(NaPO_3)_6$ becoming evident. Most conspicuous was the shifting of the optimum growth from 50 to 700 mg Mn^{++}/liter by addition of stoichiometric amounts of $(NaPO_3)_6$.

this finding suggests that the biological availability of the $(NaPO_3)_6$ complex of Mn^{++} is less than 10% of that of $MnCl_2$. Conversely, modification of the effects of $(NaPO_3)_6$ on body weight gain by manganese indicates that the toxicity of this material is probably exerted primarily through complexation of essential metals. Absorption of manganese, itself, has long been known to be inhibited by lowering of the Ca:P ratio in the diet (Wachtel, et al., 1943). However, it cannot be concluded that Mn^{++} complexation alone is responsible for the effects of $(NaPO_3)_6$ in that other essential metals, such as Fe, Mg, and Ca, are likely to be affected as well. Complexation of these metals by $(NaPO_3)_6$ would also be decreased by stoichiometric addition of Mn^{++} to $(NaPO_3)_6$ solutions particularly at the concentrations utilized in the present study. As a result of the differing latency of growth enhancement by the 700 mg Mn^{++} and 2957 mg $(NaPO_3)_6$/liter combination relative to that of 50 mg Mn^{++}/liter alone, a more general effect of $(NaPO_3)_6$ than a specific interaction with Mn^{++} seems indicated.

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